



## UWS Academic Portal

### **A characterization of the antimalarial activity of the bark of *Cylicodiscus gabunensis* Harms**

Aldulaimi, Omar; Uche, Fidelia I.; Hameed, Hamza ; Mbye, Haddijatou ; Ullah, Imran ; Drijfhout , Falko; Claridge, Timothy D.W.; Horrocks, Paul; Li, Wen-Wu

*Published in:*  
Journal of Ethnopharmacology

*DOI:*  
[10.1016/j.jep.2017.01.014](https://doi.org/10.1016/j.jep.2017.01.014)

Published: 23/02/2017

*Document Version*  
Peer reviewed version

[Link to publication on the UWS Academic Portal](#)

*Citation for published version (APA):*  
Aldulaimi, O., Uche, F. I., Hameed, H., Mbye, H., Ullah, I., Drijfhout , F., Claridge, T. D. W., Horrocks, P., & Li, W-W. (2017). A characterization of the antimalarial activity of the bark of *Cylicodiscus gabunensis* Harms. *Journal of Ethnopharmacology*, 198, 221-225. <https://doi.org/10.1016/j.jep.2017.01.014>

#### **General rights**

Copyright and moral rights for the publications made accessible in the UWS Academic Portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

#### **Take down policy**

If you believe that this document breaches copyright please contact [pure@uws.ac.uk](mailto:pure@uws.ac.uk) providing details, and we will remove access to the work immediately and investigate your claim.

# **A characterization of the antimalarial activity of the bark of *Cylicodiscus gabunensis* Harms**

Omar Aldulaimi<sup>1, 2</sup>, Fidelia I. Uche<sup>1</sup>, Hamza Hameed<sup>1</sup>, Haddijatou Mbye<sup>1</sup>, Imran Ullah<sup>1</sup>, Falko Drijfhout<sup>3</sup>, Timothy D.W. Claridge<sup>4</sup>, Paul Horrocks<sup>1</sup>, Wen-Wu Li<sup>1\*</sup>

<sup>1</sup>Institute for Science and Technology in Medicine, Keele University, Staffordshire ST5 5BG, United Kingdom

<sup>2</sup>College of Pharmacy, Al-Mustansiriyah University, Baghdad, Iraq

<sup>3</sup>Chemical Sciences Research Centre, Keele University, Staffordshire ST5 5BG, United Kingdom

<sup>4</sup>Chemical Research Laboratory, University of Oxford, Oxford OX1 3TA, United Kingdom

## **\*Corresponding authors:**

Dr. Wen-Wu Li

E-mail: w.li@keele.ac.uk

Phone: +44 (0)1782 674382

Fax: +44 (0)1782 747319

**Keywords:** *Cylicodiscus gabunensis*; malaria; gallic acid; oligosaccharide conjugates; structural elucidation.

**Abbreviations:** CG, *Cylicodiscus gabunensis* Harms; CGE, ethanol extract of CG; CGH, hexane extract of CG; CGEEA, ethyl acetate fraction of CGE; CGEBU, n-butanol fraction of CGE; CGEAQ, aqueous fraction of CGE; GC-MS, gas chromatography mass spectrometry; LC-MS, liquid chromatography mass spectrometry; NMR, nuclear magnetic resonance; TMS, trimethylsilyl; WHO, World Health Organization

## ***Abstract***

***Ethnopharmacological relevance and aim:*** A decoction of the bark of *Cylicodiscus gabunensis* Harms is used as a traditional medicine in the treatment of malaria in Nigeria. This study aims to validate the antimalarial potency of this decoction *in vitro* against *Plasmodium falciparum* and define potential bioactive constituents within the *C. gabunensis* bark.

***Materials and methods:*** A bioassay-guided separation and fractionation protocol was applied to *C. gabunensis* extracts, exploiting the use of a Malaria Sybr Green I Fluorescence assay method to monitor antiproliferative effects on parasites as well as define 50% inhibition concentrations. Spectroscopic techniques, including GC-MS, TOF LC-MS and <sup>1</sup>H NMR were used to identify phytochemicals present in bioactive fractions. Analogues of gallic acid were synthesized *de novo* to support the demonstration of the antimalarial action of phenolic acids identified in *C. gabunensis* bark. *In vitro* cytotoxicity of plant extracts, fractions and gallate analogues was evaluated against the HepG2 cell line.

***Results:*** The antimalarial activity of ethanolic extracts of *C. gabunensis* bark was confirmed *in vitro*, with evidence for phenolic acids, primarily gallic acid and close analogues such as ethyl gallate, likely providing this effect. Further fraction produced the most potent fraction with a 50% inhibitory concentration of 4.7 µg/ml. Spectroscopic analysis, including <sup>1</sup>H NMR, LC-MS and GC-MS analysis of this fraction and its acid hydrolyzed products, indicated the presence of conjugates of gallic acid with oligosaccharides. The extracts/fractions and synthetic alkyl gallate showed moderate selectivity against *P. falciparum*.

***Conclusions:*** These results support the use of the bark of *C. gabunensis* as a traditional medicine in the treatment of human malaria, with phenolic acid oligosaccharide complexes evident in the most bioactive fractions.

## 1. Introduction

Malaria imposes a significant health and socioeconomic impact within endemic regions across tropical and subtropical zones. In 2015 the World Health Organization (WHO) estimated there were some 214 million new cases of malaria globally, resulting in approximately 438,000 deaths. Of these deaths, some 90% occur within Sub-Saharan Africa (WHO, 2015). Yet over the last 15 years, the incidence of malaria and mortality figures, particularly in Africa, are falling significantly (Bhatt et al., 2015). This success follows from the introduction of interventions such as indoor residual spraying and insecticide treated bed nets, improved access to rapid diagnostic testing and the adoption of artemisinin combination therapies as the frontline antimalarial treatment. However, these successes are mirrored by increasing evidence of the development and spread of insecticide resistance and reports of artemisinin resistance within South East Asia (Ashley et al., 2014). The continued search for novel compounds with modes of action distinct to those of the current antimalarial pharmacopeia, include efforts to build on the success of plant-derived natural products, and their semi-synthetic analogues, in contributing new antimalarial leads. Traditional medicines have proven important in this search, with their importance demonstrated by the WHO which estimates that some 80% of populations in resource-poor regions depend upon these traditional medicines for antimalarial treatment.

*Cylicodiscus gabunensis* Harms (CG) (Leguminosae) is a medicinal plant used for the treatment of fever, internal abscess, jaundice and malaria by the Ibibio of the Niger Delta region of Nigeria (Okokon et al., 2006). The ethanolic stem bark extract of *C. gabunensis* shows schizontocidal activity in both early and established *Plasmodium berghei* infections in mice, with treatment showing an improved survival time comparable to that of chloroquine (Okokon et al., 2006). To date, several phytochemical studies on this plant have indicated the presence of triterpenes such as cyclicodiscic acid (Tchivounda et al., 1990), triterpenoid saponins (Mkouna et al., 2010; Tchivounda et al., 1991; Tene et al., 2011) and cyclodione (Tane et al., 1995). However, the antimalarial compounds in the plant extract are still unknown. In this study, we confirm that extracts of *C. gabunensis* possess *in vitro* inhibitory effects against the blood

stages of the human malarial parasite *P. falciparum* Dd2, using a bioassay-guided fractionation procedure to isolate fractions which were then subjected to spectroscopic methods to identify compounds with potential antimalarial activity. Our studies suggest that phenolics, such as gallic acid and its derivatives may provide the active antimalarial constituent in *C. gabunensis* bark extracts. Furthermore, it is apparent that within the most potent fractions that phenolic-oligosaccharide conjugates likely provide the source of antimalarial potency.

## **2. Materials and methods**

### *2.1 Plant materials and chemicals*

The bark of *C. gabunensis* was collected from the Imo state, Nigeria in September 2011. The plant was identified by Mr. H. Donyeachusim and a voucher specimen (UPH1028) was kept in the herbarium at the University of Port Harcourt, Nigeria. Solvents were supplied by Fischer Scientific (UK), other chemical were sourced from Sigma-Aldrich (UK).

### *2.2 Extraction of plant materials*

Powdered *C. gabunensis* bark (120 g) was macerated over 48hrs in two liters of hexane, the process repeated and the filtrates combined and evaporated at 30°C under vacuum to yield the hexane extract (CGH, 0.9 g, 0.75%). The residue was extracted again by maceration using the same procedure with 70% ethanol; the filtrates were combined and dried at 45°C under vacuum to give the ethanol extract (CGE, 11.3 g, 9.4 %).

### *2.3. Fractionation and separation of bioactive compounds*

CGE (11 g) was dissolved in 700ml deionized water and partitioned with ethyl acetate and n-butanol sequentially to give the ethyl acetate fraction (CGEEA, 0.9g), butanol fraction (CGEBU, 8.3g) and aqueous fraction (CGEAQ, 1.6g). The CGEBU fraction was further fractionated on a silica gel glass column (35-75 µm; 4×60 cm) washed with solvent system CHCl<sub>3</sub>/MeOH/H<sub>2</sub>O (10: 1: 0.1) with increasing methanol (final concentration 100%) at a flow rate of 3 ml/min to give 10 pooled fractions (CGEBU-F(1-

10)). Further separation of CGEBU-F10 was performed using preparative HPLC, yielding 8 sub-fractions CGEBU-F10-(1 to 8). A full description of the preparative HPLC is provided in the Supplementary materials.

#### 2.4 Spectroscopic and chemical analysis

A full description of acid hydrolysis method, gas chromatography mass spectrometry (GC-MS), liquid chromatography mass spectrometry (LC-MS) and proton nuclear magnetic resonance ( $^1\text{H}$  NMR) spectroscopic analysis are provided in the Supplementary materials.

#### 2.5 Chemical synthesis of gallic acid derivatives

Gallic acid and other phenolic acids were purchased from Sigma-Aldrich (UK). In addition, five gallic acid derivatives (Table 1) were prepared *de novo* for this study. Their synthesis and spectroscopic analysis is described in the Supplementary materials.

#### 2.6. *In vitro* determination of antimalarial activity

The *in vitro* antimalarial activities of the plant extracts, fraction or pure compounds were assessed against intraerythrocytic *P. falciparum* culture (Dd2 strain) using the Sybr Green 1 Fluorescence assay as described (Smilkstein et al., 2004) and modified (Hasenkamp et al., 2013). CGE and its three fractions CGEEA, CGEBU, CGEAQ; CGH; and CGEBU-F1-10 were prepared as three concentrations in DMSO (100, 33.3, and 11.1  $\mu\text{g/ml}$ ) with DMSO not greater than 1% of the total concentration, while the most active fractions CGEBU-F10 and CGEBU-F10-7 were prepared using a serial two-fold dilution (200, 100, 50, 25, 12.5, 6.25, 3.13, 1.56, and 0.78  $\mu\text{g/ml}$ ). Normalized fluorescence signals were determined against controls with 1% DMSO (100% growth) and exposure to a supralethal 10 $\mu\text{M}$  dose of chloroquine (0% growth). Determination of the 50% inhibitory concentration ( $\text{IC}_{50}$ ) was carried out with analysis of a log transformed concentration versus normalized fluorescence effect curve using GraphPad Prism software (v5.0) on data prepared from three independent biological replicates.

#### 2.7. *In vitro* cytotoxicity against HepG2 cells

The *in vitro* activities of the plant extracts, fraction or pure compounds were assessed against HepG2 (human liver carcinoma) using a modified alamar blue fluorescence assay as described (Horrocks et al,

2013). HepG2 cells were maintained in DMEM media supplemented with 10% (v/v) foetal bovine serum and 0.2% (v/v) of a penicillin (10 U/ml)/streptomycin (10 µg/ml) solution at 37°C in an atmosphere of 5% CO<sub>2</sub>. 100 µL of cell suspension of HepG2 cells were seeded into a 96-well microplate at a density of  $1 \times 10^5$  cells/ml. After 24h of growth at 37°C, 100 µl of extracts or compounds in medium were added to the microplate wells, starting at either 150mM or 150µg/ml, to provide a twofold dilution series. Untreated controls were provided by cultures exposed to a 1% DMSO (100% growth) with a drug-treated control provided by exposure to 500nM actinomycin D (0% growth). The microplate was incubated at 37°C for a further 48 hrs before the cellular reductase activity was measured using an alamar blue<sup>®</sup> cell viability reagent (ThermoFisher). 20 µL of alamar blue was added into each well and mixed by shaking the plate. The fluorescence signal was measured at 570 nm using a Glomax multi-detection System after 6 hours incubation. The 50% cytotoxicity concentration (CC<sub>50</sub>) was determined by analysis of a log transformed concentration versus normalized fluorescence signal curve using GraphPad Prism software (v5.0).

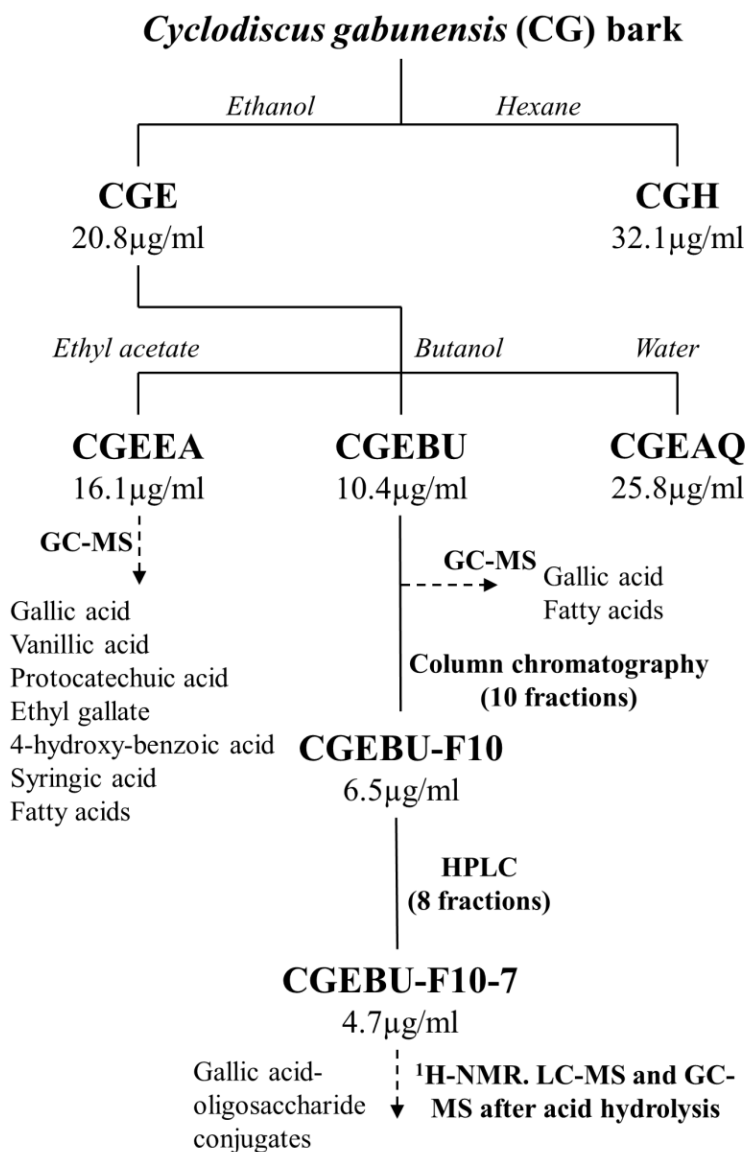
### **3. Results**

#### *3.1. Bioassay guided separations of extract of *Cylicodiscus gabunensis* bark*

A sample of *C. gabunensis* bark was extracted sequentially into hexane and 70% ethanol to produce CGE and CGH extracts. The anti-proliferative activity of these extracts was determined using *in vitro* growth inhibition assays of the Dd2 strain (chloroquine resistant) of *P. falciparum*, Chloroquine was used as a positive control (Table 1). Given the apparently greater antimalarial activity of CGE (IC<sub>50</sub> 20.8 µg/ml), further fractional separation in ethyl acetate (CGEEA), butanol (CGEBU) and water (CGEAQ) was undertaken. Here, the CGEBU fraction showed an approximate two-fold increase in antimalarial action over CGE (IC<sub>50</sub> 10.4µg/ml). The CGEBU fraction was subjected to column chromatography to yield 10 sub-fractions, with fraction CGEBU-F10 the most potent with an IC<sub>50</sub> of 6.5µg/ml. CGEBU-F10 was further separated using preparative HPLC (Figure S1) to yield eight sub-fractions. Of these, CGEBU-F10-



7 was the most active, providing an  $IC_{50}$  of 4.7  $\mu\text{g/ml}$ . Figure 1 shows a summary of the bioassay fractionation strategy employed. The cytotoxicity of CGE, CGEBU and CGEBU-F10 decreased in turn, with concurrent increases in antiplasmodial activity, although the selectivity (Selective index, SI  $CC_{50}/IC_{50}$ ) against HepG2 cells only reached a modest value of >23. Insufficient CGEBU-F10-7 was available for cytotoxicity assays.



**Figure 1.** Schematic illustrating the bioassay guided fractionation of *C. gabunensis* bark, indicating key fractionation methods and the phytochemical identified. The solvents used in the initial separation steps

are indicated using italics, with the  $IC_{50}$  of derived fractions indicated below each. Further column chromatography (generating 10 pooled fractions) of CGEBU and HPLC fractionation (8 pooled fractions) of CGEBU-F10 were used to derive the most potent fraction, CGEBU-F10-7. Where indicated with dotted lines, the spectroscopic methods listed were used to identify the key phytochemical components.

In order to identify potentially bioactive compounds, GC-MS was initially applied to both CGEEA and CGEBU fractions after trimethylsilylation (Johnson-Ajinwo et al., 2015). These analyses (Figures S2 and S3; Table S1 and S2), indicate that 3,4,5-trihydroxybenzoic acid (gallic acid) as well as several fatty acids were present in both the CGEEA and CGEBU extracts, but also that additional benzoic acid derivatives, including 3-methoxy-4-hydroxybenzoic acid (vanillic acid, 0.4%), 3,4-dihydroxybenzoic acid (protocatechuic acid, 2%), and 3,4,5-trihydroxybenzoic ethyl ester (ethyl gallate, 2%), were also present in CGEEA.

Independent confirmation of the potency of these benzoic acid derivatives was provided through additional growth inhibition assays of *P. falciparum* using commercially sourced materials (Table 1). These results indicated that either loss of a phenol group (e.g. vanillic acid) or substitutions to phenol groups of gallic acid (e.g. 3-methoxy-4-hydroxybenzoic acid or 3,4,5-trimethoxybenzoic acid) led to a decrease in antimalarial potency. Of note was the similar potency of ethyl gallate and propyl gallate to gallic acid, although these esterified derivatives are significantly less hydrophilic based on their predicted partition coefficients (LogP) (Table 1). To further explore this structure activity relationship, five additional esters were synthesized (Table 1, Table S3 and S4, Figure S4, and Supplementary materials), using gallic acid as a precursor, and their *in vitro* potency determined. These data indicate that whilst most have an  $IC_{50}$  comparable to that of ethyl gallate or propyl gallate, lauryl gallate, the most lipophilic derivative, was much lower at 2.2  $\mu$ M, some 6-7 fold less than that of gallic acid. Unfortunately, the selectivity of these gallic acid derivatives remained only modest at best (Table 1) with alkyl and alkenyl gallates showed the best selectivity with selective indices between 10 and >17.

**Table 1.** *In vitro* antiparasmodial activity (IC<sub>50</sub>) and cytotoxicity (CC<sub>50</sub>) against HepG2 cells of extracts, fraction, gallic acid and its derivatives (along with standard deviation, SD, based on three biological repeats). Names of commercially sourced compounds or those synthesized (marked with \*, Supplementary material) for this study are listed. Selective index, SI, is calculated as CC<sub>50</sub>/IC<sub>50</sub>. The LogP of compounds was predicted using Chemdraw software (PerkinElmer, USA). nd, not determined.

Extract/fraction/compound	IC <sub>50</sub> ± SD	CC <sub>50</sub> ± SD	SI	Log P
	(µg/ml)	(µg/ml)		
CGE	20.8 ± 1.8 (µg/ml)	59.8±14 (µg/ml)	2.9	nd
CGEBU	10.4 ± 1.9 (µg/ml)	50.1±5.2 (µg/ml)	4.8	nd
CGEBU-F10	6.5 ± 0.6 (µg/ml)	> 150 (µg/ml)	>23	nd
CGEBU-F10-7	4.7 ± 0.4 (µg/ml)	nd	nd	nd
	(µM)	(µM)		
Gallic acid	26.8 ± 4.4	11±1	0.4	0.42
3,4-Dihydroxybenzoic acid	83.4 ± 10.0	> 150	>1.8	0.81
3-Methoxy-4-hydroxybenzoic acid	71.2 ± 11.0	> 150	>2.1	1.08
3,4,5-Trimethoxybenzoic acid	112.3 ± 15.2	> 150	>1.3	1.21
Ethyl gallate	9.3 ± 3.1	> 150	>16.1	1.02
Propyl gallate	24.5 ± 2.9	> 150	>6.1	1.51
Propan-2-yl gallate*	13.9 ± 1.8	> 150	>10.8	1.34
2-Methylpropyl gallate*	16.9 ± 2.3	78±20	4.6	1.91
2-Methylbutyl gallate*	11.8 ± 1.2	38±10	3.2	2.33
3-Methylbut-2-en-1-yl gallate*	17.5 ± 2.2	87± 16	5.0	1.92
3-Methylbutyl gallate*	8.8 ± 1.5	> 150	>17.0	2.26
Lauryl gallate	2.2 ± 0.2	30±2	13.6	5.27
Chloroquine	0.17 ± 0.02	nd	nd	5.00
Actinomycin D	nd	0.0038±0.0013	nd	3.80

### 3.2. Structural elucidation of the phytochemical content of CGEBU10-7

GC-MS analysis of trimethylsilyl (TMSi) derivatives of CGEBU-F10-7 did not produce resolvable spectra, suggesting that the masses of the phytochemicals present were greater than that readily discernable using this approach. This was confirmed using an alternative LC-MS approach that revealed a complex mixture of compounds with masses ranging between 1000 and 1500 Da (Figure S5). Thus, the CGEBU-F10-7 fraction was subjected to acid hydrolysis, with GC-MS of the TMSi derivatives of these hydrolysed products carried out (Figure S6). This approach revealed the presence of gallic acid as well as three pairs of isomeric monosaccharides:  $\alpha$ - and  $\beta$ -L-arabinose;  $\alpha$ - and  $\beta$ -L-rhamnose; and  $\alpha$ - and  $\beta$ -L-glucose. It was considered likely that the acid hydrolysis procedure led to the anomerization of these sugars, which was subsequently confirmed in a subsequent experiment using commercially sourced sugar standards in water at 100°C. In addition,  $^1\text{H}$  NMR (Figure S7) of CGEBU-F10-7 was carried out, revealing multiple peaks in the regions of  $\delta$  6-8 ppm and  $\delta$  3-6 ppm, supporting the existence of aromatic and sugar moieties in this complex fraction. In particular, two doublets at  $\delta$  5.2 (d,  $J$  = 3.7 Hz) and  $\delta$  4.4 (d,  $J$  = 7.6 Hz) would support the presence of anomeric protons of  $\alpha$ -L-arabinosyl and  $\beta$ -D-glucopyranosyl moiety, respectively (Tene et al., 2011). Together, these data suggest the presence of gallic acid – oligosaccharide conjugates within the CGEBU-F10-7 fraction.

## 4. Discussion

Using a comprehensive bioassay-guided fractionation and isolation of the bark of *C. gabunensis* we provide evidence for the presence of antimalarial activity of both crude extracts, correlating with the previous observation of activity of an ethanolic extract against the murine malaria *P. berghei* (Okokon et al., 2006), but also refine this activity further to demonstrate specific activity of phenolic acids present in CGEBU and CGEEA. A preliminary structure activity relationship study of benzoic acid and gallate derivatives, including several synthesized *de novo* for this study, indicate that free hydroxyl groups on the aromatic ring for gallic acid and gallate esters are essential for their antimalarial effect. Gallic acid and protocatechuic acid have both previously been shown to moderate antimalarial activities against

chloroquine-resistant *P. falciparum* strain W2 (Garcia-Alvarez et al., 2013) with an additional study using ethyl gallate reporting an *in vitro* antiplasmodial effect of IC<sub>50</sub> between 9 - 35  $\mu$ M (Ramanandraibe et al., 2008). Results from these previous studies were in agreement with our own, with our study further suggesting that alkyl gallates, in particular lauryl gallate, have a greater antiplasmodial activity than gallic acid itself (Table 1). These promising observations, however, are moderated by the apparent restricted selectivity of these phenolic acids as compared to the HepG2 cell line.

Bioassay guided fractionation gave a HPLC fraction (CGEBU-F10-7) which was most active. LC-MS, GC-MS and <sup>1</sup>H NMR analysis suggested that conjugates of oligosaccharide with gallic acid may be present in this fraction. However, the exact linkage between the monosaccharides and gallic acid needs further detailed study. Previously an oligosaccharide derivative (a resin glycoside, cryptophilic acid C) from *Scrophularia cryptophila* showed antimalarial activity with IC<sub>50</sub> value of 4.2  $\mu$ g/ml (Tasdemir et al., 2008). Ellagic acid (a dimer of gallic acid) and a galloyl glycoside from *Tristaniopsis calobuxus* bark extract had IC<sub>50</sub> values of 0.5 and 3.2  $\mu$ M, respectively (Verotta et al., 2001). 1-O-galloyl-6-O-luteoyl- $\alpha$ -D-glucose from *Phyllanthus niruri* also showed very low IC<sub>50</sub> (1.4  $\mu$ g/ml) (Subeki et al., 2005). Together with our own data, these observations suggest that a galloyl moiety within the bark of *C. gabunensis* likely supplies the observed antimalarial activity of this traditional medicine.

## 5. Conclusions

Bioassay guided fractionation of *C. gabunensis* extract yielded fractions with increasing antimalarial activity and selectivity of action. Using spectroscopic and chemical methods, gallic acid, ethyl gallate and benzoic acids were demonstrated to be present and likely contributed to this antimalarial activity. The likely bioactive component in this traditional medicine for the treatment of malaria is a galloyl glycoside conjugate.

## Acknowledgments

We are grateful for Ph.D. scholarships to Mr. Omar Aldulaimi and Ms. Fidelia Uche from MOHSER/IRAQ, ETF Nigeria, respectively. We also thank Bridging the Gaps MRC Centenary Award (WWL and PH).

## Appendix A. Supplementary information

Supplementary data associated with this article can be found in the online version.

## References

- Ashley, E.A., Dhorda, M., Fairhurst, R.M., Amaratunga, C., Lim, P., Suon, S., Sreng, S., Anderson, J.M., Mao, S., Sam, B., Sopha, C., Chuor, C.M., Nguon, C., Sovannaroeth, S., Pukrittayakamee, S., Jittamala, P., Chotivanich, K., Chutasmit, K., Suchatsoonthorn, C., Runchaoen, R., Hien, T.T., Thuy-Nhien, N.T., Thanh, N.V., Phu, N.H., Htut, Y., Han, K.T., Aye, K.H., Mokuolu, O.A., Olaosebikan, R.R., Folaranmi, O.O., Mayxay, M., Khanthavong, M., Hongvanthong, B., Newton, P.N., Onyamboko, M.A., Fanello, C.I., Tshefu, A.K., Mishra, N., Valecha, N., Phyoo, A.P., Nosten, F., Yi, P., Tripura, R., Borrmann, S., Bashraheil, M., Peshu, J., Faiz, M.A., Ghose, A., Hossain, M.A., Samad, R., Rahman, M.R., Hasan, M.M., Islam, A., Miotto, O., Amato, R., MacInnis, B., Stalker, J., Kwiatkowski, D.P., Bozdech, Z., Jeeyapant, A., Cheah, P.Y., Sakulthaew, T., Chalk, J., Intharabut, B., Silamut, K., Lee, S.J., Vihokhern, B., Kunasol, C., Imwong, M., Tarning, J., Taylor, W.J., Yeung, S., Woodrow, C.J., Flegg, J.A., Das, D., Smith, J., Venkatesan, M., Plowe, C.V., Stepniewska, K., Guerin, P.J., Dondorp, A.M., Day, N.P., White, N.J. Tracking Resistance to Artemisinin Collaboration (TRAC), 2014. Spread of artemisinin resistance in *Plasmodium falciparum* malaria. *New Engl J Med* 371, 411-423.
- Bhatt, S., Weiss, D.J., Cameron, E., Bisanzio, D., Mappin, B., Dalrymple, U., Battle, K.E., Moyes, C.L., Henry, A., Eckhoff, P.A., Wenger, E.A., Briët, O., Penny, M.A., Smith, T.A., Bennett, A., Yukich, J., Eisele, T.P., Griffin, J.T., Fergus, C.A., Lynch, M., Lindgren, F., Cohen, J.M., Murray, C.L., Smith, D.L.,

Hay, S.I., Cibulskis, R.E., Gething, P.W., 2013. The effect of malaria control on *Plasmodium falciparum* in Africa between 2000 and 2015. *Nature* 526, 207-211.

Garcia-Alvarez, M.C., Moussa, I., Soh, P.N., Nongonierma, R., Abdoulaye, A., Nicolau-Travers, M.L., Fabre, A., Wdzieczak-Bakala, J., Ahond, A., Poupat, C., Ikhiri, K., Benoit-Vical, F., 2013. Both plants *Sebastiania chamaelea* from Niger and *Chrozophora senegalensis* from Senegal used in African traditional medicine in malaria treatment share a same active principle. *J Ethnopharmacol* 149, 676-684.

Hasenkamp, S., Sidaway, A., Devine, O., Roye, R., Horrocks, P., 2013. Evaluation of bioluminescence-based assays of anti-malarial drug activity. *Malar J* 12, 58.

Horrocks, P., Pickard, M.R., Parekh, H.H., Patel, S.P., Pathak, R.B., 2013. Synthesis and biological evaluation of 3-(4-chlorophenyl)-4-substituted pyrazole derivatives. *Org Biomol Chem* 11, 4891-4898.

Johnson-Ajinwo, O.R., Richardson, A., Li, W.W., 2015. Cytotoxic effects of stern bark extracts and pure compounds from *Margaritaria discoidea* on human ovarian cancer cell lines. *Phytomedicine* 22, 1-4.

Mkouna, P., Tiabou, A.T., Kouam, J., 2010. Triterpenoid Derivatives from *Cylicodiscus gabunensis*. *Chem Pharm Bull* 58, 1100-1102.

Okokon, J.E., Ita, B.N., Udokpoh, A.E., 2006. Antiplasmodial activity of *Cylicodiscus gabunensis*. *J Ethnopharmacol* 107, 175-178.

Ramanandraibe, V., Grellier, P., Martin, M.T., Deville, A., Joyeau, R., Ramanitrahasimbola, D., Mouray, E., Rasoanaivo, P., Mambu, L., 2008. Antiplasmodial phenolic compounds from *Piptadenia pervillei*. *Planta Med* 74, 417-421.

Smilkstein, M., Sriwilaijaroen, N., Kelly, J.X., Wilairat, P., Riscoe, M., 2004. Simple and inexpensive fluorescence-based technique for high-throughput antimalarial drug screening. *Antimicrob Agents Chemother* 48, 1803-1806.

Subeki, S., Matsuura, H., Takahashi, K., Yamasaki, M., Yamato, O., Maede, Y., Katakura, K., Kobayashi, S., Trimurningsih, T., Chairul, C., Yoshihara, T., 2005. Anti-babesial and anti-plasmodial compounds from *Phyllanthus niruri*. *J Nat Prod* 68, 537-539.

- Tane, P., Bergquist, K.E., Tene, M., Ngadjui, B.T., Ayafor, J.F., Sterner, O., 1995. Cyclodione, an unsymmetrical dimeric diterpene from *Cylicodiscus gabunensis*. *Tetrahedron* 51, 11595-11600.
- Tasdemir, D., Brun, R., Franzblau, S.G., Sezgin, Y., Calis, I., 2008. Evaluation of antiprotozoal and antimycobacterial activities of the resin glycosides and the other metabolites of *Scrophularia cryptophila*. *Phytomedicine* 15, 209-215.
- Tchivounda, H.P., Koudogbo, B., Besace, Y., Casadevall, E., 1990. Cylicodiscic Acid, a dihydroxy pentacyclic triterpene carboxylic-acid from *Cylicodiscus gabunensis*. *Phytochemistry* 29, 3255-3258.
- Tchivounda, H.P., Koudogbo, B., Besace, Y., Casadevall, E., 1991. Triterpene saponins from *Cylicodiscus gabunensis*. *Phytochemistry* 30, 2711-2716.
- Tene, M., Chabert, P., Note, O., Kenla, T.J.N., Tane, P., Lobstein, A., 2011. Triterpenoid saponins from *Cylicodiscus gabunensis*. *Phytochem Lett* 4, 89-92.
- Verotta, L., Dell'Agli, M., Giolito, A., Guerrini, M., Cabalion, P., Bosisio, E., 2001. In vitro antiplasmodial activity of extracts of *Tristanopsis* species and identification of the active constituents: ellagic acid and 3,4,5-trimethoxyphenyl-(6'-O-galloyl)-O-beta-D-glucopyranoside. *J Nat Prod* 64, 603-607.
- World Health Organization. World Malaria Report 2105. <http://www.who.int/malaria/publications/world-malaria-report-2015/en>